Preparation of Chemically Competent *E. coli* -- Rubidium Chloride Method

transfer to centrifuge tubes and ice for 20-30 min

1. Inoculate a single colony from a rich plate (Luria-Bertani agar) into 5 ml of rich broth (Luria-Bertani; LB) in a test tube. Shake overnight at 37°C.

2. Subculture the overnight 1:100 in LB+20 mM $MgSO_4$ (typically 2.5 ml culture into 250 ml of LB + 5 ml 1M $MgSO_4$). Grow to OD590=0.4-0.6 or Klett=60 (~2-3 h). ~~(O.D. 0.25-0.4 in plate reader - 200ul sample)~~

3. Centrifuge ~~4,000 rpm~~ 3220 g 10 min at 4 C. Use 50ml conical tubes. Divide culture evenly into 8 tubes. Eppendorf Centrifuge 5810R - 15 amp version. Swinging bucket rotor.

4. Gently resuspend pellet in 1/2.5 Volume Unit ice cold TFBI. For 250 ml subculture, use 100 ml TFBI. 300 120
   1. discard supernatant
   2. add 25 ml TFBI to 4 of the tubes
   3. resuspend using the 25ml autopipetter
   4. transfer cells to tubes without TFBI and resuspend

   You now have 4 tubes of cells.

Keep all steps on ice and chill all pipets, tubes, flasks, etc. from this point on.

5. Incubate on ice for 5 min.

6. Centrifuge ~~4,000 rpm~~ 3220 g 10 min 4°C

7. Resuspend pellet in 1/25 original volume cold TFB2. For 250 ml of original subculture, use 10 ml TFB2. 300 12
   1. discard supernatant
   2. add 10 ml TFB2 to one of the tubes
   3. resuspend
   4. transfer cells to next tube and resuspend - repeat this step until all 4 tubes have been resuspended

8. Incubate on ice 15-60 min. before aliquoting for storage at -70°C.

9. While incubating, get a tray of ice, label the microfuge tubes (top), and let them chill.

10. Aliquot 110ul and 220ul - about half and half.

11. Quick-freeze the tubes using an EtOH/dry ice bath.

12. Store in -80C freezer.

After the tubes are well-frozen they can be dumped loose into a box or ice-cream carton, or transferred to slots in a storage box. Be careful not to get alcohol on the lips of the tubes. Liquid nitrogen can also be used.

This procedure works with most strains and should routinely give > 107 cfu/ug of pBR322 with reasonably healthy K-12 derivatives (using 0.1 ng/transformation). Frozen cells last at least a year.

Recipes

RB (Luria-Bertani medium)

per liter:
10 g Tryptone (Difco)
5 g Yeast Extract (Difco)
5 g NaCl
2 ml 1N NaOH

250mL Culture
0.294 g KOAc Potassium Acetate
1.2092 g RbCl
0.111 g $CaCl_2$
0.99 g $MnCl_2 \cdot 4H_2O$
15 mL Glycerol

opt to 100mL

100mL culture
0.72 g KOAc
2.97 g RbCl
0.273 g $CaCl_2$
1.548 g $MnCl_2$
↓
60 mL → 9 mL Glycerol

TFBI

1.2g 30 mM KOAc (potassium acetate)
4.95g 100 mM RbCl
0.455g 10 mM $CaCl_2$
2.58g 50 mM $MnCl_2$
15% glycerol

$30 \frac{mmol}{L} * \frac{1 mol}{1000 mmol} * 0.41L * 98.14 g/mol = 1.2 g$ KOAC
$100mM * \frac{1 mol}{1000 mM} * 0.41L * 120.92 g/mol = 4.95 g$ RbCl
$10 mM CaCl_2 * \frac{1}{1000} * 0.41L * 110.99 g/mol = 0.455 g CaCl_2$
$50 mM MnCl_2 * \frac{1}{1000} * 0.41L * 125.84 g/mol = 2.58 g MnCl_2$

Adjust to pH 5.8 with acetic acid and filter (0.45 um, Nalgene units or Millipore filters) to sterilize.

It is convenient to make this as:

5 g	RbCl (Alfa)
12.3 ml	KOAc 1 M
4.1 ml	$CaCl_2$ 1 M
20.5 ml	$MnCl_2$ 1 M (this is pink)
61.5 ml	glycerol
pH to 5.8 with	8 ml HOAc 0.1 M

~~1.25g RbCl~~

make up to 410 ml; distribute in 100 ml sterile aliquots; and use 1 aliquot/250 ml culture.
60 100

TFBII

10 mM MOPS or PIPES
1.25g 75 mM $CaCl_2$
0.18g 10 mM RbCl
15% glycerol

$10 * \frac{1}{1000} * 0.15 * 209 = 0.31 g$ MOPS
$75 \frac{mmol}{L} * \frac{1 mol}{1000 mmol} * 0.150L * 110.99 g/mol = 1.25 g CaCl_2$
$10 mM RbCl * \frac{1}{1000} * 0.15 * 120.92 g/mol = 0.18 g$ RbCl

Adjust pH to 6.5 with KOH and filter to sterilize

Make up as

1.5ml	MOPS 1 M pH 6.5 (this is yellow)
11.25ml	$CaCl_2$ 1 M
1.5ml	RbCl 1M
22.5g mL	glycerol
pH with	1 N KOH

make to 150 ml; filter; use 10 ml per original 250 ml culture.

Procedure adapted from the John Innes Institute (Norwich, England) via Joseph Utermohlen (Univ. of Arizona).

This procedure improves transformation with most strain backgrounds, when compared with $CaCl_2$ procedure.